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Chapter 11

Antiviral and Immunomodulatory Activities of Ascorbic Acid

Raxit J. Jariwalla and Steve Harakeh

1. INTRODUCTION

It has been known since the early days of ascorbic acid research that the appearance of scurvy, which is caused by deficiency of this vitamin, is associated with decreased resistance to infection (Reid and Briggs, 1953). Over the years, it has become well recognized that ascorbate can bolster the natural defense mechanisms of the host and provide protection not only against infectious disease, but also against cancer and other chronic degenerative diseases. The functions involved in ascorbate's enhancement of host resistance to disease include its biosynthetic (hydroxylating), antioxidant, and immunostimulatory activities. In addition, ascorbate exerts a direct antiviral action that may confer specific protection against viral disease. The vitamin has been found to inactivate a wide spectrum of viruses as well

Abbreviations used in this chapter: AZT, 3′-azidothymidine; CD, cluster of differentiation; CHS, Chediak-Higashi syndrome; Con A, concanavalin-A; EAE, experimental allergic encephalomyelitis; HIV, human immunodeficiency virus; HTLV-1, human T-cell leukemia virus-1; IL, interleukin; INF, interferon; NAC, N-acetyl cysteine; NF-kB, neurotrophic factor-k beta; PBMC, peripheral blood mononuclear cells; PMA, phorbol 12-myristate 13-acetate; PMN, polymorphonuclear neutrophil; RT, reverse transcriptase; TNF-2 tumor necrosis factor alpha.

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as suppress viral replication and expression in infected cells. In this article we review the antiviral and immunostimulatory effects of ascorbate and their relevance to control of acute and chronic viral infections. Detailed discussion of the biosynthetic activities of ascorbate has been presented in a review by Englard and Seifter (1986). The antioxidant function of ascorbate has been reviewed recently by Bendich (1988).

2. INHIBITION OF VIRAL INFECTIVITY BY ASCORBATE

2.1. Inactivation of Virus Activity *in Vitro*

Ascorbic acid has been demonstrated to inactivate both DNA- and RNA-containing viruses that infect hosts ranging from bacteria to humans. The first account of an antiviral effect was made within two years following the vitamin's discovery, when Jungeblut reported that crystalline vitamin C could inactivate the ability of poliomyelitis virus to produce paralysis. Virus inactivation was evident at concentrations of ascorbate that can be attained in the body through supplementation. Shortly after this initial finding, Jungeblut and other investigators published a succession of reports showing that ascorbate caused inactivation of a wide spectrum of viruses *in vitro,* including herpes simplex, vaccinia, foot-and-mouth disease, rabies, tobacco mosaic, and bacterial viruses (early reports cited in Stone, 1972).

The mechanism of virus inactivation was investigated by Murata and coworkers who utilized DNA and RNA bacteriophages as a model (Murata and Kitagawa, 1973; Murata and Uike, 1976). They found that all types of phages were sensitive to ascorbate inactivation, with loss of infectivity occurring at different rates for different phages. Virus inactivation was shown to be dependent upon the presence of oxygen and was enhanced by the addition of copper, which catalyzes oxidation of ascorbic acid to form reactive hydroxyl radicals. The virus inactivation process could be prevented by the addition of free radical scavengers.

In the bacteriophage studies, the nucleic acid within the virion was identified as the target of ascorbate action, since it was shown to undergo strand breakage during the inactivation process. It was concluded that free radicals generated during the autooxidation of ascorbic acid mediated inactivation of virus infectivity through damage to viral nucleic acid. This conclusion is consistent with subsequent studies demonstrating degradation of phage/viral nucleic acids by ascorbate and ascorbate plus hydrogen peroxide in the presence of trace amounts of copper ions (Kazakov *et al.,* 1988). Whether such a prooxidant mechanism operates *in vivo* has yet to be demonstrated, although it is well known that ascorbate, hydrogen peroxide, and copper are involved in the metabolism of aerobic cells (Halliwell and Gutteridge, 1984; Weitberg, 1987).

In their studies, Murata and Kitagawa (1973) did not detect ascorbate-mediated damage of phage protein. However, more recent studies carried out in our labora-

tory with a human retrovirus, HIV, showed that ascorbate caused inactivation of its virion-associated enzyme, reverse transcriptase, upon prolonged incubation of virus particles *in vitro* (see below).

2.2. Inhibitory Effects on Intracellular Virus Replication

Aside from the inactivation of cell-free virus *in vitro,* ascorbate has been shown to inhibit the growth and expression of virus in laboratory cultures of infected cells. The first report of ascorbate-mediated inhibition of viral growth was made in cell cultures infected with rhinovirus. Ascorbate, in concentrations nontoxic to host cells, interfered with progressive multicycle replication of rhinovirus in cultures of WI-38 human fibroblasts (Schwerdt and Schwerdt, 1975). The authors did not determine the mechanism of the inhibitory effect, although they ruled out the involvement of interferon.

Further investigations of ascorbate inhibition of virus replication have been carried out using retroviruses as models. Bissell *et al.* (1980), working with the avian retrovirus of chickens, found that while cell-free virus was resistant to ascorbate inactivation upon short-term treatment *in vitro,* exposure of virus-infected cultures to the vitamin resulted in reduction of virus replication and lowered infectivity of newly replicated virus. A subsequent study found that ascorbate interfered with the replication and cell-transforming potential of Rous sarcoma virus by stabilizing the differentiated state of chicken cells (Schwarz, 1991). In a lymphocytic cell line latently infected with human T-cell leukemia virus (HTLV-1), ascorbate was shown to interfere with virus production triggered by chemical inducers added to the culture medium (Blakeslee *et al.*, 1985).

More recent studies from our laboratory have examined the antiviral action of ascorbate on HIV (Harakeh *et al.*, 1990; Harakeh and Jariwalla, 1991). In initial studies, cultures of chronically infected T-lymphocytic cells were grown in the presence of nontoxic ascorbate concentrations (25–150 μg/ml or 0.14–0.85 mM). The results indicated a dose-dependent suppression of HIV as determined from the levels of virion-associated reverse transcriptase (RT) and p24 core protein recovered in the culture supernatant. At the highest nontoxic dose of ascorbate tested (150 μg/ml or 0.85 mM), extracellular RT activity was reduced by over 99% and p24 antigen level was decreased by ~90% after four days of incubation. Exposure of an acutely infected CD4+ T-cell line to 0.14–0.57 mM ascorbate led to dose-dependent suppression of giant-cell (syncytia) formation, with 90% inhibition occurring at the highest ascorbate dose. Incubation of cell-free virus with 0.57–0.85 mM ascorbate for short periods (18–24 hr at 37°C) had no detectable effect on viral RT activity or syncytial-forming ability. However, prolonged exposure of virus (four days at 37°C) to 0.57–0.85 mM ascorbate resulted in a 70–93% decline in RT activity as compared to a 96% to >99% reduction in RT activity of virus released from chronically infected cells. In the same studies, exposure of chronically infected cells to 0.57–0.85 mM ascorbate for 18 hr was found to reduce extracellular RT

titer by 78–89% compared to untreated control. These data indicated two distinct effects of ascorbate: one seen after overnight incubation involving intracellular inhibition of a component or step in virus replication and the other detected after prolonged exposure. The second effect involved the suppressive action of ascorbate on both viral production in infected cells and RT stability in extracellular viral particles.

In more recent studies, the effect of ascorbate was investigated in latently infected cell lines that had been stimulated with tumor promoter or inflammatory cytokine to trigger virus production (Harakeh and Jariwalla, 1994, and unpublished data). Pretreatment of cells with 100–300 μg/ml ascorbate followed by cell stimulation with phorbol ester (PMA) or cytokine (TNF-α) resulted in dose-dependent suppression of virus activation. Unlike N-acetyl cysteine (NAC), which suppressed cytokine-stimulated HIV expression through inhibition of transcriptional activation by NF-kB, ascorbate seemed to have no effect on the activity of this transcription factor. AZT, a known inhibitor of *de novo* infection, had no effect on virus production in either unstimulated chronically infected cells or in stimulated latently infected cells (Harakeh and Jariwalla, 1994).

The molecular mechanism by which ascorbate suppresses HIV is just beginning to be understood (Jariwalla and Harakeh, 1992; Harakeh *et al.*, 1994). Although it has not been ruled out that viral nucleic acid is targeted during replication in acute infection, studies in chronically infected cells that contain integrated HIV have revealed that newly transcribed viral RNA is not the target of ascorbate inactivation. Thus, the size of RNA molecules made in ascorbate-treated cells was similar to those seen in untreated cells. Furthermore, although the levels of viral RNA were increased twofold over an internal cellular RNA marker in ascorbate-treated compared to untreated cells, this difference was biologically unimportant because it did not translate into a correspondingly higher level of viral protein. The amount of HIV protein synthesized in ascorbate-treated cells was found to be similar to that in untreated controls. These results suggested a posttranslational mechanism of ascorbate-mediated inhibition of HIV.

Analysis of viral enzyme activity in the presence and absence of ascorbate indicated that reverse transcriptase was a common target of ascorbate action on HIV. Thus, in our studies on HIV, RT activity was reduced in virus released from chronically and latently infected cells as well as in virus preparations incubated with ascorbate over a prolonged period (Harakeh *et al.*, 1990, 1994; Harakeh and Jariwalla, 1991). This inhibitory effect appears to be highly selective because other viral enzymes or reporter proteins such as HIV *tat* or bacterial CAT were not inactivated in cells treated with ascorbate (Harakeh and Jariwalla, 1994). It is not presently known whether this ascorbate effect is due to selective modification or cleavage of susceptible sites in the viral enzyme. The results on HIV are consistent with a hypothesis of ascorbate-mediated effect specific to protein inactivation that needs to be further investigated by site-directed analysis of viral enzyme as a model protein.

2.3. Ascorbate Effects on Viral Infections *in Vivo*

The pathological or clinical symptoms produced by viruses shown to be inactivated by ascorbate *in vitro* can be alleviated upon administration of ascorbate *in vivo* (Stone, 1972; Pauling, 1986).

Early observations made on the control of viral diseases by ascorbic acid involved poliomyelitis, viral hepatitis, measles, mumps, chicken pox, shingles, infectious mononucleosis, viral encephalitis, and influenza. These early papers are detailed in the monograph by Stone (1972). More recently, successful prophylaxis was reported by Morishige and Murata (1978). Reporting on their observations with 1537 patients who had undergone blood transfusions following surgery, they found that the incidence of hepatitis B was 7% among 170 patients who received little or no vitamin C and less than 0.2% (three cases of non-B) in 1367 who received 2–6 g or more per day after whole blood transfusion.

The effect of vitamin C on the common cold has been a controversial subject. Pauling (1971a,b) reviewed four placebo-controlled trials that had been carried out prior to 1971 and concluded that vitamin C was effective in lowering the incidence and severity of this condition. Since the maximum benefit had been seen in studies by Ritzel (1961, 1976) using a dose of 1g/day, Pauling proposed that such amounts of vitamin C would be beneficial in the prevention and treatment of the cold.

Although Pauling's announcement was greeted with skepticism in the medical community, it stimulated a large number of clinical studies. Recently, Hemilä (1994) reviewed the data from 21 placebo-controlled trials carried out since 1971 in which vitamin C had been used in amounts greater than or equal to 1g/day. By applying a method of binomial distribution analysis, Hemilä found that although there were large variations in the benefits observed, nevertheless in each of the 21 studies, vitamin C produced a significant decline in morbidity involving a reduction in the duration of episodes and the severity of the symptoms of the common cold by an average of 23%. In that analysis, there was no consistent evidence that vitamin C reduced the incidence of the common cold in persons ingesting 1–3 g per day.

The lack of vitamin C effect on incidence of colds in the studies analyzed by Hemilä (1994) may be related to the small amount of vitamin tested (Pauling, 1986). Thus, Cathcart has found that the maximum concentration of vitamin C tolerated by persons increases with illness and severity of disease. Based on initial observations made with 9000 patients who had been treated with large doses of vitamin C, Cathcart (1981) reported that while healthy persons can tolerate oral intakes from 4–15 g per day, their bowel-tolerance level—the amount slightly lower than that which produces a laxative effect—increased to values of 30–60 g during a mild cold, 60–100 g during a severe cold, and greater than 100 g during an attack of influenza or flu. In cases of severe viral illnesses such as infectious mononucleosis or pneumonia, the bowel-tolerance doses were found to approach 200 g per day. Cathcart found that vitamin C therapy for the common cold and infectious diseases was most effective when administered at the bowel-tolerance dose. Upon control of

the disease, the tolerance limit was found to return to the normal range. Based on such observations, he concluded that a 100 g cold cannot be treated successfully with a few grams of vitamin C. Since all double-blind trials of the common cold have utilized low to moderate doses of vitamin C, there is a need to test large dosages (approaching bowel tolerance) to see whether a greater alleviating effect occurs and whether the incidence of the cold is affected significantly.

In addition to treating persons with the common cold and the flu, Cathcart (1981) has successfully applied his orthomolecular treatment of titrating to bowel tolerance to treat other acute as well as chronic viral conditions with megadoses of vitamin C, including hepatitis (30–100 g), viral pneumonia (100–200 g), infectious mononucleosis (100–200 g), and other herpes virus infections, such as herpes simplex and varicella zoster (i.e., shingles). His observations are consistent with earlier reports of Klenner (1971, 1974), showing that comparable megadoses of vitamin C were effective in controlling both viral and bacterial infections.

During the early years of the AIDS epidemic, Cathcart also monitored patients who had voluntarily ingested large doses of vitamin C and treated a small number of AIDS patients with 50 to 200 g of oral and intravenous ascorbate. From his initial observations, he concluded that vitamin C ameliorated the symptoms of AIDS and reduced the frequency and severity of opportunistic infections (Cathcart, 1984). By 1989 he had treated over 250 HIV-positive patients with similar findings (Cathcart, 1990).

The biochemical basis for prevention and alleviation by ascorbate of symptoms associated with viral infections may depend not only on its antiviral action, but also on other effects of the vitamin. In the case of rhinoviral infection associated with the common cold and influenza virus–induced infection in mice, pathologic changes do not result primarily from direct cytopathic effects of replicating virus, but seem to correlate with toxic effects of reactive oxygen radicals generated during the process of neutrophil activation and inflammation (Maeda and Akaike, 1991; Hemilä, 1992). There is evidence for increased utilization of vitamin C during the common cold infection and prevention of its loss in supplemented patients (Hemilä, 1992). Overreaction of the host immune response has also been implicated in the pathology of other viral diseases, including lymphocytic choriomeningitis in mice, viral hepatitis, dengue fever, herpesviral disease, and HIV infection (Maeda and Akaike, 1991). Because ascorbate is a potent antioxidant (Bendich, 1988; see also Chapter 17), supplementary vitamin C may provide cell protection by neutralizing extracellular and leaky oxidants generated during immune activation. While this reducing action of vitamin C may explain its beneficial effect, other functions of ascorbate may also be involved in its protective effect against viral infections.

3. IMMUNOMODULATORY EFFECT OF ASCORBATE

Aside from its direct antiviral and antioxidant action, ascorbate may afford additional protection against viral diseases through an effect on the immune system.

A role in immunomodulation was first suggested because lymphoid cells can accumulate ascorbate to concentrations at least 50 times higher than that present in blood plasma. There is much evidence in the literature that supports stimulation by ascorbate of both humoral and cell-mediated immune responses. Below we review the available data supporting an immunomodulatory role for the vitamin.

3.1. Ascorbate Influence on Function of Phagocytes

The high intracellular accumulation of ascorbate in neutrophils and mononuclear phagocytes and its decrease during disease and trauma indicates that vitamin C may play a role in phagocytic function (Muggli, 1993). Many studies in the literature show that ascorbate can influence motility and chemotaxis of phagocytes. In addition, the antioxidant ability of ascorbate provides cellular protection to phagocytes from oxidative self-damage caused by free radicals without interfering with their formation during the process of respiratory burst (Muggli, 1993). The specific role of ascorbate in the life cycle of phagocytic leukocytes merits discussion.

Phagocytosis involves the engulfing, ingestion, and destruction of microorganisms by phagocytic leukocytes. The latter include polymorphonuclear neutrophils (PMNs), eosinophils, and monocytes-macrophages that have the ability to ingest and destroy microorganisms.

3.1.1. Effects on Neutrophil Adhesion and Chemotaxis

An essential step in phagocytosis involves travel of neutrophils to the site of infection through their adherence to the vessel wall and travel between endothelial cells (diapedesis). Jonas *et al.* (1993) reported that ascorbate reduced neutrophil adhesion to endothelial cells and provided a protective effect; this conclusion was not valid because the difference between ascorbate-treated and control groups was only 1%. In another report, on seven patients who had renal transplant (Thorner *et al.*, 1983), ascorbate was shown to increase neutrophil adhesion. More studies with larger sample size are needed to elucidate further the role of ascorbate in adhesion.

Many other studies have investigated the effect of ascorbate on the directed movement (chemotaxis) of neutrophils to the site of infection. Chemotactic response, as well as random migration of phagocytes, is enhanced by incubation with millimolar concentrations of ascorbic acid *in vitro* (Anderson and Theron, 1979; Goetzl *et al.*, 1974; Goetzl, 1976; Dallegri *et al.*, 1980; Boxer *et al.*, 1979). Smith and Walker (1980) reported that ascorbate at 2.5 μM could also enhance neutrophil chemotaxis, although the significance of this is unclear.

Physiological significance of *in vitro* studies utilizing millimolar amounts of ascorbate is supported by data from studies in humans, who cannot synthesize vitamin C, as well as in animals that can make their own ascorbate (Anderson, 1981a). In a small study of five healthy human subjects, Anderson *et al.* (1980) showed that the daily ingestion of 2–3 g sodium ascorbate for one week led to a

significant stimulation of neutrophil mobility. Other studies claimed that ascorbate did not affect chemotaxis of neutrophils. For instance, Vogel *et al.* (1986) noted that in dental students ascorbate had no effect on neutrophil chemotaxis; however, control volunteers had ascorbate intake two or more times above the recommended daily allowance. In a recent report, Johnston *et al.* (1992) showed that ascorbate supplementation was associated with enhancement of chemotaxis in normal volunteers. The observed effect did not correlate with plasma ascorbate levels and was attributed to an indirect action of ascorbate mediated through detoxification of histamine (see Chapter 9).

Other researchers used neutrophils from sick patients and studied the effect of ascorbate on migration and chemotaxis. Neutrophil mobility was enhanced by ascorbate in patients with recurrent bacterial infections (Anderson and Theron, 1979), defects in bacterial killing (Rebora *et al.*, 1980), impaired chemotaxis associated with tuberculosis (Gatner and Anderson, 1980), and trauma victims (Maderazo *et al.*, 1986). Similar results were reported in persons with genetic disease of phagocytic function. Thus, Anderson (1981b) found that the administration of 1 g of sodium ascorbate to children suffering from chronic granulomatous disease and defective neutrophil mobility led to an increase in PMN leukocyte mobility. Boxer *et al.* (1976) studied the effect of ascorbate on Chediak-Higashi syndrome (CHS), which is another phagocytic disease. They reported that PMN leukocyte chemotaxis was increased by administering 200 mg ascorbic acid for two months to patients suffering from CHS. However, it was noted that daily intake of the vitamin was necessary, or else the enhancement was reversed.

3.1.2. Effect on Respiratory Burst

Upon stimulation of neutrophils by bacteria or chemical stimuli, oxygen uptake associated with a respiratory burst is initiated. Ascorbate has been shown to increase the activity of the hexose monophosphate shunt in resting and stimulated neutrophils, leading to the synthesis of NADPH (DeChatalet *et al.*, 1972). The latter process is involved in (i) reduction of molecular oxygen to superoxide anion (O_2^-) via the activity of NADPH oxidase and (ii) regeneration of oxidized glutathione (GSSG) to the reduced form (GSH), catalyzed by glutathione reductase in the presence of dehydroascorbate. It is not presently known which of these two reactions is affected by ascorbate. Although it has been argued (Muggli, 1993) that ascorbate does not participate as a reducing agent in the formation of bactericidal oxygen radicals, further investigation is necessary before a firm conclusion can be reached.

3.1.3. Neutralization of Harmful Extracellular Oxidants

Activated phagocytes can release superoxide anion into the extracellular fluid as well as leak it into the cytoplasm. Superoxide can give rise to hydrogen peroxide

and hydroxyl radicals. Thus, phagocytic cells need to be protected from cellular damage caused by extracellular and intracellular oxidants generated as by-products of phagocytosis. Since neutrophils have a short life span after phagocytosis, it has been suggested that ascorbate is needed mainly to maintain cell viability during phagocytosis and not to prevent cell death (Oberritter *et al.*, 1986). In contrast, macrophages exhibit a relatively long life span and thus require protection from damaging effects of oxidants at all phases, including the postphagocytic phase. Ascorbate may provide cell protection, as its normal levels are twofold higher in macrophages than in neutrophils and monocytes (Schmidt and Moser, 1985) and macrophages increase their consumption of ascorbate during macrophage phagocytosis (Oberritter *et al.*, 1986).

Furthermore, it has been shown (Anderson *et al.*, 1987; Anderson and Lukey, 1987) that ascorbate can mediate efficient neutralization of extracellular phagocyte-derived oxidants without affecting the formation of bactericidal oxygen radicals within the intracellular phagosome. This appears to be a major role of ascorbate in phagocytic cells. In addition, ascorbate was shown to protect rabbit macrophages *in vitro* from injuries associated with phagocytosis (McKee and Myrvik, 1979).

3.2. Effects on Lymphocyte-Mediated Immune Responses

Lymphocyte performance is known to be affected by ascorbate concentration. For instance, Cameron *et al.* (1979) reported an enhancement in immunocompetence upon saturation of lymphocytes with ascorbate. Both *in vitro* and *in vivo* studies have indicated an immunostimulatory effect of ascorbate on lymphocytic cells (references cited in Jariwalla and Harakeh, 1994).

3.2.1. Studies in Experimental Animals

In most studies on vitamin C and the immune response, guinea pigs have been used as a model because, like humans, they do not synthesize vitamin C endogenously. Vitamin C was shown to be required for regeneration of lymphoid tissue in both mice and guinea pigs following irradiation with X-rays. Although it was suggested that vitamin C may work through modulation of the activity or production of thymic humoral factors (Dieter, 1971), the specific thymic factors involved have not been identified. In another study, ascorbate was reported to be needed for the differentiation of lymphoid organs during the development of cockerel and young rats stressed with steroids (Dieter and Breitenbach, 1971). The relevance of these observations to normal development needs to be investigated.

The role of vitamin C during immunization was also studied. It was shown that guinea pigs placed on a scorbutic diet after immunization with attenuated *Mycobacterium butryicum* responded to intradermal injection of tubercular protein. On the other hand, animals made scorbutic prior to the tuberculin inoculation did not respond positively to the tuberculin (Mueller and Kies, 1962; Zweiman *et al.*,

1966). When these animals were replenished with ascorbic acid, their normal tuberculin response was restored (Mueller and Kies, 1962). These results indicated that vitamin C was required during the induction rather than the expression of delayed-type hypersensitivity reactions (Mueller and Kies, 1962). It has also been shown that in guinea pigs reared on an ascorbate-deficient diet, the induction of experimental allergic encephalomyelitis (EAE), caused by the injection of CNS tissue homogenized in Freund's complete adjuvant, was markedly inhibited by vitamin C deprivation (Mueller *et al.*, 1962). In this case, the restoration of ascorbic acid levels did not reverse the effects of EAE. Ascorbic acid deprivation of guinea pigs was also shown to prolong skin graft survival (Kalden and Guthy, 1972).

The relationship of the above observations in ascorbate-deprived guinea pigs to lymphocyte dysfunction is not clear-cut. Observations by Fraser *et al.* (1978) on lymphocytes from scorbutic animals suggested a decrease in T lymphocyte count and an increase in B lymphocytes. This phenomenon was reversed when animals were supplemented with a high dose (250 mg/day) of ascorbate. The scorbutic animals had a lower T cell response to concanavalin A (Con A) and an increased B cell response to lipopolysaccharide, compared to animals on diets replenished with ascorbic acid. However, Anthony *et al.* (1979) did not observe a reduction in T cells in the spleen of scorbutic animals. Because vitamin C–deficient diets can lead to inanition and scorbutic animals are more susceptible to infections, better models are needed to address the relationship between direct effects of vitamin C level and lymphocytic function (Cunningham-Rundles *et al.*, 1993).

Alternatively, indirect effects of vitamin C may be involved. Thus, vitamin C has been reported to regenerate vitamin E from the tocopherol free radical (Packer *et al.*, 1979; Bendich *et al.*, 1984), suggesting that the main effect of vitamin C may lie in its effect on vitamin E levels. Others (Oh and Nakano, 1989) have shown that ascorbic acid can detoxify histamine resulting from the activation of lymphocytes *in vitro*. Johnston and Huang (1991) indicated that ascorbate caused a decrease in histamine levels in guinea pigs. Since histamine is a biphasic regulator of lymphocyte transformation, ascorbate detoxification of the same may explain both activating and suppressive effects of the vitamin on lymphocyte response.

3.2.2. Human Studies

In healthy volunteers ingesting one to several grams a day of ascorbic acid, an enhancement in lymphocyte blastogenesis was observed following *in vitro* exposure to pokeweed mitogens or lectins (Oh and Nakano, 1989; Yonemoto *et al.*, 1976; Yonemoto, 1979). The amount of vitamin C administered directly influenced the degree of lymphocyte blastogenesis (Anderson *et al.*, 1980; Siegel and Morton, 1977; Delafuente and Panush, 1979). Studies by Siegel and Morton (1977) indicated a similar increase in T-lymphocyte blastogenesis to Con A following ascorbate supplementation. Also, Panush *et al.* (1982) showed an enhancement in T-lymphocytic response to viral infections upon the ingestion of several grams of

ascorbate per day in healthy individuals. In another study the ingestion of ascorbate was shown to inhibit the induction of supressor activity in human volunteers (Anderson *et al.*, 1983).

In contrast to the above studies, no increase in proliferation response to mitogen was seen in peripheral blood lymphocytes isolated from healthy elderly persons taking large doses of vitamin C along with other vitamins (Goodwin and Garry, 1983). The same persons, however, did show improved reactivity in a skin test.

3.2.3. *In Vitro* Studies

Cultures of human lymphocytes supplemented with ascorbate potentiated mitogen or lectin-induced mononuclear DNA synthesis (Delafuente and Panush, 1979; Manzella and Roberts, 1979; Panush and Delafuente, 1979). In contrast, Goodwin and Garry (1983) reported that ascorbate treatment *in vitro* inhibited lymphocyte proliferation in response to mitogen stimulation. A dose-dependent inhibitory effect of ascorbate (0.4–6.0 mg/dl) was recently described by Cunningham-Rundles *et al.* (1993) in a standardized microtiter cell assay of peripheral blood mononuclear cells exposed to a broad range of concentrations of phytohemagglutinin. A similar inhibitory effect was seen when Con A was used to activate mononuclear cells *in vitro*. Pokeweed mitogen gave much less inhibition. However, when peripheral blood mononuclear cells (PBMC) were stimulated with influenza A antigen, cellular proliferation was enhanced. This effect was verified using PBMC cultures from a sequential series of 15 normal donors; of these, eight responded positively to influenza A antigen *in vitro* and seven failed to respond. Ascorbate did not affect lymphocyte proliferation of nonresponders to influenza A antigen but significantly augmented the proliferation rate of antigen responders.

Cunningham-Rundles *et al.* (1993) also examined the effect of vitamin C on lymphocyte proliferation response to interleukin 2 (IL-2) and gamma interferon (INF-γ). They found that low levels of vitamin C (2.5–4.0 μg/ml) suppressed proliferation response to IL-2, suggesting a basis for the vitamin's inhibitory effect on mitogen-induced lymphocyte proliferation. In contrast, vitamin C enhanced the proliferative response to INF-γ without affecting its production. The latter result may explain the augmenting influence of vitamin C on proliferative response to influenza A antigen. From these data, the authors concluded that at least some interaction of vitamin C with the immune response may involve the specific regulation of the cytokine network.

3.3. Other Effects on the Immune Response

Ascorbate seems to affect the immune response at more than one level. Aside from distinct roles in stimulating phagocytic function and lymphocyte activation, large doses of ascorbate have also been found to stimulate production of protein molecules involved in the immune response. Earlier reports conflicted on ascor-

bate's ability to enhance antibody responses and stimulate the esterase component of complement (Thomas and Holt, 1978). However, recent studies have given unequivocal results. By using standardized immune reagent ratios and a constant level of circulating ascorbate in guinea pigs, Feigen *et al.* (1982) demonstrated that large doses of vitamin C enhanced antibody production and provided protection against systemic anaphylaxis. Similarly, using indirect and direct methods for measuring complement in ascorbate-deprived guinea pigs compared to pair-fed controls, Johnston and coworkers (1985, 1987) showed a significant relationship between ascorbate level and production of complement C1q. In these newer studies, it was shown conclusively that dietary increase of ascorbate resulted in tissue-saturating levels of ascorbate and in a 30-fold increase in complement C1q level compared to animals fed normal or suboptimal levels of vitamin C. These observations were confirmed and extended in a more recent study (Haskell and Johnston, 1991).

Dietary vitamin C was also shown to enhance interferon production by the mouse after stimulation with murine leukemia virus (Siegel, 1973; Siegel and Morton, 1977). In separate studies, ascorbate also demonstrated enhanced interferon production in mouse (Siegel, 1975) and human embryonic fibroblasts (Dahl and Depre, 1976; Karpinska *et al.*, 1982) exposed *in vitro* to inducers of interferon. Since ascorbate has been shown to regulate the activity of γ interferon on lymphocytic cultures, this is a most important avenue for further study.

4. CLINICAL APPLICATION TO VIRAL AND IMMUNODEFICIENCY DISEASES

It is clear from the foregoing sections that vitamin C exerts pronounced antiviral and immunomodulatory effects that should have potential value in the control of viral and immune-system diseases. Although much information exists on the control of viral diseases with supplemental ascorbate (Section 2.3), little data has been reported on the effect of vitamin C supplementation on T-cell responses in immunosuppressed persons. One clinical study reported on measles in native children of South Africa, which is associated with a high death rate caused by secondary infections (Joffe *et al.*, 1983). Administration of a few grams of vitamin C per day to these patients during the convalescent phase resulted in the restoration of their T-cell counts to normal levels. In children suffering from Chediak-Higashi disease, supplementation with vitamin C restored their immune systems (Baehner, 1980). In a recent Canadian study conducted on elderly people, it was shown that supplementation with a multivitamin and multimineral formulation (containing 80 mg of vitamin C) improved their immune responses and reduced the levels of infection in those subjects (Chandra, 1992).

To date, clinical studies have been carried out using small doses of ascorbate, whereas preliminary observations from noncontrolled studies suggest that large doses provided strong benefits. Large-scale trials are needed to demonstrate conclusively the value of megadoses of vitamin C *in vivo*. From a practical standpoint,

it may be difficult to carry out controlled studies because of ethical reasons involved in withholding a harmless substance such as vitamin C from the placebo group. An alternate solution may be to compare the efficacy of large doses of ascorbate with low to moderate doses in the same group of patients. This may be achieved by a stepwise bowel titration protocol where the patients serve as their own controls. Such trials are being designed to test vitamin C efficacy in HIV-infected persons.

5. CONCLUSIONS

In summary, ascorbate has been shown to (1) inactivate a broad spectrum of viruses *in vitro,* (2) suppress replication of rhino and retroviruses in cultures of infected cells, and (3) confer protection against viral diseases *in vivo*. Viral nucleic acid and virion-associated enzyme have been identified as specific components sensitive to inhibition by ascorbate. Inactivation of bacteriophage infectivity has been linked to degradation of its genomic DNA or RNA caused by free radicals generated during the autooxidation of ascorbate. Retroviral suppression by ascorbate has been attributed to an indirect stabilization of the cellular differentiated state as well as to a direct antiviral effect involving selective impairment of viral enzymatic activity. The precise molecular mechanisms underlying these effects are not presently known. In addition to its antiviral action, ascorbate exerts antioxidant and immunostimulatory effects, providing additional protection against viral infections *in vivo*. As an antioxidant, ascorbate can neutralize efficiently both extracellular and intracellular (leaky cytoplasmic) oxidants resulting from neutrophil activation and inflammatory reactions. As an immunomodulator, ascorbate affects both cell-mediated and humoral immune responses that include enhancement of neutrophil adhesion, chemotaxis and respiratory burst, regulation of antigen-induced lymphocyte proliferation and cytokine function, enhancement of delayed-type hypersensitivity reactions, and elevation of antibody, complement C1q, and interferon production. Controlled clinical trials to date of ascorbate against viral infections have used low to moderate doses of the vitamin. Since pharmacologic functions of ascorbate may become manifest at large doses, additional studies are needed to establish conclusively the optimum value of this multifunctional nutrient against viral and immunosuppressive diseases.

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